

HETEROCHROMATIN IN MAMMALS,
WITH SPECIAL REFERENCE TO
ERINACEUS AND MUSTELA

by

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LUND 1979

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By due permission of the Faculty of Science
of the University of Lund,
to be publicly defended,
together with the papers listed overleaf,
in the lecture hall of the Institute of Genetics, Lund
on Friday, November 30, 1979 at 1 p.m.
for the degree of Doctor of Philosophy.

The thesis will be defended in English.

This thesis is based on the following papers:

- I. MANDAHL, N. 1978. Variation in C-stained chromosome regions in European hedgehogs (Insectivora, Mammalia). - Hereditas 89:107-128
- II. MANDAHL, N. 1979. Localization of nucleolar organizing regions in European hedgehogs (Insectivora, Mammalia). - Hereditas 91:149-161
- III. FREDGA, K. and MANDAHL, N. 1973. Autosomal heterochromatin in some carnivores. - Nobel Symp. 23:104-117
- IV. MANDAHL, N. and FREDGA, K. 1975. Q-, G- and C-band patterns of the mink chromosomes. - Hereditas 81:211-220
- V. MANDAHL, N. and FREDGA, K. 1980. A comparative chromosome study by means of G-, C- and NOR-bandings of the weasel, the pygmy weasel and the stoat (Mustela, Carnivora Mammalia). - Hereditas 93, in press

References to these papers will be made by the above Roman numerals



the era of chromosome banding.

The introduction of a great variety of new chromosome staining techniques during the last decade has been of extraordinary importance for the elucidation of a large number of problems in the field of cytogenetics (for reviews see HSU 1973; SCHNEDL 1973; TRILLIUX and LEJEUNE 1975; EVANS 1977; JORGENSEN et al. 1978; SCHNEDL 1978). For most types of bandings several alternative methods are available and the joint work of quite a number of scientists has resulted in simplified and highly reproducible methods, suitable for routine use. Thus, the refinement by which cytogenetical analysis can be performed today has been greatly enhanced.

The Q-, G- and R-staining techniques are extremely useful for the identification of chromosomes (and parts of them), thus facilitating the determination of the chromosome segments involved in structural rearrangements. Consequently, these techniques have been eagerly applied within the fields of comparative mammalian cytogenetics, cancer research, human clinical genetics, gene mapping and many others.

In comparative chromosome studies should be carried out without the application of the C-staining technique, which has proven useful for the identification of constitutive heterochromatin. Qualitative subdivision of such heterochromatin may be achieved by applying modified methods, as the C_d - and Giemsa-11 stainings. Furthermore, qualitative heterogeneity of the positively C-stained regions can also be demonstrated by scoring their differential staining reaction to different banding techniques (cf. III; SCHNEDL 1973; JALAL et al. 1974). Also quantitative variation has been found to be a widespread characteristic of constitutive heterochromatin. Chromosome variants, as demonstrated by Q- and C-staining, occur frequently, in man actually to such an extent that no two individuals (except monozygotic twins) are expected to be identical in this respect. Thus, the use of these techniques has led to the realization that chromosomal polymorphisms are far more

common than was thought before the days of chromosome banding. Selective staining of facultatively heterochromatinized rodent X chromosomes is also feasible (KANDA and YOSIDA 1979).

Differential staining techniques based on incorporation of BrdU into the DNA have several important applications, e.g. for studies of the replication patterns and demonstration of sister chromatid exchanges. It is true that autoradiographic techniques for such purposes were available before the banding era, but the modern techniques utilizing BrdU are less laborious and give a higher resolution in most cases. Studies of SCE have developed into a simple and sensitive test method for screening of suspected mutagenic/carcinogenic agents.

Another set of methods, allowing selective staining of the nucleolar organizing regions, has yielded important knowledge concerning the localization and activity of ribosomal genes. The failure to identify inactive ribosomal genes is a shortcoming that must be borne in mind when using these methods. The in situ hybridization technique, utilizing radioactively labelled ribosomal RNA, identifies all sites of ribosomal genes but gives no information about their activity. Thus, these two techniques are mutually complementary.

Finally, several base pair specific DNA binding fluorochromes and base specific anti-nucleoside antibodies have been used to localize G-C and A-T rich chromosome regions and to characterize different types of constitutive heterochromatin.

Progress in molecular biology.

Some years before the chromosome banding era was initiated, as well as during the time the banding techniques evolved explosively, advances in nucleic acid biochemistry gave rise to a whole battery of new procedures. These include DNA reassociation kinetics, ultracentrifugation in isopycnic gradients, in situ hybridization and cleavage of DNA by restriction enzymes. As a matter of fact the first Giemsa banding technique (C-staining) arose as a by-product

from experiments in this field. The application of chromosome banding and biochemical procedures has gone hand in hand, the molecular data providing a physical basis for the banding results. On the other hand the banding techniques allow a more or less coarse, but simple identification of molecular characteristics. In particular, close relationship has been established between highly repetitive DNA sequences and constitutive heterochromatin, as demonstrated by the C-staining. The concordance is, however, not complete (for discussion see JOHN and MIKLOS 1979; KURNIT 1979).

Amount and distribution of constitutive heterochromatin in mammals.

The total amount, distribution and size of constitutive heterochromatin in individual chromosomes, is liable to considerable variation in different mammals. By now a great variety of mammalian species have been investigated with C-staining. Since heterochromatin of sex chromosomes is outside the scope of the present study this discussion will be limited to autosomal heterochromatin.

The total length of dark C-bands varies widely between species belonging to different genera but also between species within a genus. Low quantities are frequently found for example in felids (PATHAK and WURSTER-HILL 1977), and in the opossum, Didelphys virginiana, all autosomes were reported to be devoid of C-bands (SINHA et al. 1972; SINHA and KAKATI 1976), whereas for example cetaceans and some mustelids have many and relatively large C-bands (ÄRNASON 1974b; III; V). Obvious differences in the amount between species of the same genus may be exemplified by the deer mouse, genus Peromyscus, and the genus Mustela (PATHAK et al. 1973; GRAFODATSKY et al. 1977). P. crinitus has 8 heterochromatic chromosome arms and P. eremicus as many as 48, whereas the lowest amount in Mustela is found in M. lutreola and the highest in M. altaica, the differences being mainly due to the presence or absence of heterochromatic short arms. In both cases, species with intermediate amounts of heterochromatin exist. In 5 hamster species, all belonging to different genera, the amount of heterochromatin varies from 13% in Mystromys albicaudatus to 34% in

Cricetus cricetus and Mesocricetus auratus (GAMPERL et al. 1978). Although the two latter species have the same amount, the distribution differs markedly, being predominantly centromeric in C. cricetus but found in several short arms in M. auratus.

The distribution of constitutive heterochromatin in mammalian chromosomes was characterized in the early days of C-staining by HSU and ARRIGHI (1971). On the basis of a restricted number of species they concluded that heterochromatin occurs frequently in the centromeric regions, less frequently in terminal regions and only occasionally in interstitial positions, and these conclusion still hold true after a much greater number of species have been studied. The screening of 133 species, from the literature, characterized by C-staining, showed that 54 had bands only in the centromeric regions, though some of them did not have bands in all centromeres. The C-bands were of quite varying sizes, some of the extending into one or both chromosome arms. Only 3 species were reported to completely lack centromeric bands, viz. Didelphys virginiana having no autosomal C-bands (SINHA et al. 1972), Felis nigripes having several terminal and one interstitial band and Felis margarita having only terminal bands and some chromosomes totally devoid of heterochromatin (PATHAK and WURSTER-HILL 1977)

Varying numbers of centromeric bands were present in 130 species terminal in 72 (including the 29 species with wholly heterochromatic chromosomes or chromosome arms) and interstitial bands in 36 species. Considering the number of chromosomes instead of species would drastically increase the number of centromeric positions the terminals to some extent and the interstitials only insignificantly. Interstitial C-bands often appear close to secondary constrictions in the mustelids (IV; V) and some pinnipeds (ÁRNASON 1974a). Perhaps this is a common type of interstitial bands. Besides being few in number, interstitial bands are frequently relatively small, the cetaceans and the antelope squirrels being spectacular exceptions (ÁRNASON 1974b; MASCARELLO and MAZIRIMAS 1977).

Interspecific variation of constitutive heterochromatin.

Within the same family or genus, some species may display grossly dissimilar karyotypes, others practically identical ones; the level of karyotypic similarity not necessarily being correlated with the phenotypic traits. Thus, for example, the felids show a striking karyotypic uniformity, although G-banding studies have demonstrated more diversification than previously thought (WURSTER-HILL and MAY 1973), whereas the Indian muntjac and Reeves' muntjac, though quite similar in appearance, have extremely different karyotypes (WURSTER and BENIRSCHKE 1970). The latter situation also holds true for two species of Callicebus (BENIRSCHKE and BOGART 1976).

Numerical and morphological changes of mammalian karyotypes are commonly brought about by Robertsonian translocations and pericentric inversions, respectively. Another major mechanism for karyotype evolution, involving redistribution, addition or loss of constitutive heterochromatin, has been postulated (SCHMID 1967; JOPP 1969). Chromosome studies by C-staining have shown this postulate to be highly relevant, and have also led to revision of the interpretation of the mechanism for intra- and interspecific chromosome variation in Peromyscus (DUFFEY 1972; BRADSHAW and HSU 1972; THAK et al. 1973). With a few exceptions, the differences were due to varying numbers of heterochromatic short arms and not to pericentric inversions as thought before. Since then, several cases of karyotype dissimilarities between related species have been found to consist only, or chiefly, of differences in the amount and/or distribution of heterochromatin, as in Erinaceus, Carollia, Protoris, Lemur, Mus, Neotoma and Rattus (I; STOCK 1975; HARADA and SIDA 1978; SASAKI et al. 1975; MARKVONG et al. 1975; MASCARELLO and HSU 1976; SHARMA and GADI 1977). In other cases, karyotype evolution has proceeded by both structural rearrangements and changes in heterochromatin pattern, as in some Peromyscus, Dipodomys, Notomys, Mustela, Equus and some bovids (ARRIGHI et al. 1976; HATCH et al. 1976; BAVERSTOCK et al. 1977b; V; RYDER et al. 1978; EVANS et al. 1973).

When related species display varying amounts of constitutive heterochromatin it may be difficult to decide the evolutionary direction of changes, whether gain or loss. By comparative chromosome banding studies in Mustela, GRAFODATSKY et al. (1976, 1977) arrived at the conclusion that in this genus gain of heterochromatin was more likely. In another case, Myotis bats, evidence from the geographical distribution indicated that addition of heterochromatin step by step, associated with an inversion in one chromosome pair, was the most probable evolutionary pathway (HARADA and YOSIDA 1978). Furthermore, data from hopping mice, genus Notomys, are also well compatible with addition of heterochromatin (BAVERSTOCK et al. 1977a). Strong evidence for evolutionary elimination of constitutive heterochromatin at the interspecific level is hard to find in the literature.

A striking phenomenon reappears frequently in related species differing in heterochromatic constitution, namely, the fact that a particular change is often not restricted to just a single autosome pair but affects several, or all of them. In Mustela, M. erminea has no heterochromatic arms whereas, M. nivalis and M. altaica have 7 pairs with heterochromatic arms (III; V; GRAFODATSKY et al. 1977); in Peromyscus (2n=48) the number of heterochromatic short arms varies from 8 in P. crinitus to 48 in P. eremicus, although leucopus and P. maniculatus have some euchromatic short arms resulting from pericentric inversions (PATHAK et al. 1973; ARRIGHI et al. 1976; MURRAY and KITCHIN 1976). Heterochromatic short arms also appear in a varying number of chromosomes in different species of Neotoma and in most autosomes of Mus dunni in contrast to M. musculus (MASCARELLO and HSU 1976; MARKVONG et al. 1975). In the closely related species Rattus blanfordi and R. cutchicus the former has markedly larger centromeric C-bands in all chromosomes (SHARMA and GADI 1977).

Furthermore, some cetaceans have large interstitial blocks of heterochromatin in several pairs which is an uncommon situation in mammals (ÄRNASON 1974b). One chromosome race of Uromys caudimaculatus differs from another race and also from the related genus Melomys by several terminal heterochromatic blocks as described

low (BAVERSTOCK et al. 1976, 1977a). Repeated centric fusions are found in Swiss and Italian populations of mice, thus giving rise to chromosome numbers of 26 and 22, respectively, instead of 20 (GROPP et al. 1972; CAPANNA et al. 1975). The presence of such a common trend of chromosomal evolution in a group of animals has been termed karyotypic orthoselection by WHITE (1973).

Interspecific variation of constitutive heterochromatin.

Although significant intraspecific variation in C-band pattern is expected is less common than interspecific variation, it is by no means rare. It should be particularly rewarding to search for such variation in species with taxonomic problems, including species that are subdivided into a large number of subspecies.

One of the most drastic differences is found within the Australian mid rodent Uromys caudimaculatus, collected from two different regions of rain forest in Queensland (BAVERSTOCK et al. 1976). The northern chromosome race appears with 12 pairs of mostly large terminal and 2 pairs of interstitial C-bands, whereas the southern chromosome race have no substantial heterochromatic regions in the autosomes, but instead a varying number of large to intermediate heterochromatic B-chromosomes. In Peromyscus maniculatus different subspecies and populations display varying numbers of heterochromatic short arms (DUFFEY 1972; BRADSHAW and HSU 1972; MURRAY and KITCHIN 1976). Structural and quantitative differences involving heterochromatic regions were found within Neotoma species (MASCARELLO and WARNER 1974; MASCARELLO and HSU 1976) and quantitative variation was also observed between two subspecies of Mus musculus (DEV et al. 1975). Furthermore, in a variety of subspecies of Rattus rattus, YOSIDA and SAGAI (1975) found different patterns of heterochromatin distribution. Their data indicated that the evolutionary changes of heterochromatin in black rats have proceeded by diminution and disappearance.

Within each of the European hedgehogs, Erinaceus europaeus and E. europaeus, differences in number and distribution of large terminal blocks of heterochromatin are found in specimens from dif-

ferent geographical regions (I). The pattern of heterochromatin distribution does not, however, correlate completely with the existing division into subspecies based on internal and external morphology.

Intraindividual variation of constitutive heterochromatin.

Size variation of constitutive heterochromatin between homologous chromosomes seems to be ubiquitous as judged from the numerous examples given in the literature. In 1971 CRAIG-HOLMES and SHAW, and LUBS and RUDDLE drew attention to the high incidence of heterochromatin polymorphism in human chromosomes. Since then, C-staining in different groups of mammals has shown that such polymorphism is a widespread and regular phenomenon, although manifested more or less conspicuously. Indeed, the frequent occurrence of polymorphism must be considered a main characteristic of constitutive heterochromatin.

The incidence and mode of heterochromatin variation is hardly known in feral mammals since usually only single or few specimens are studied. Frequency studies and analysis of quantitative data have been performed more extensively in humans than in any other mammals (CRAIG-HOLMES et al. 1973; MCKENZIE and LUBS 1975; MÜLLER et al. 1975; BUCKTON et al. 1976; GHOSH and SINGH 1976; LUBS et al. 1977; THARAPEL and SUMMIT 1978); to some extent such information is now also available in European hedgehogs (I). In man, it is especially the large heterochromatic regions in the long arms close to the centromere of chromosomes 1, 9 and 16 that have been noted for size variation. Both plus and minus variants have been found at high rates, and the frequencies may differ among ethnological groups. No difference in the frequencies of extreme variants in Nos. 1, 9 and 16 was found between white and black children from the U.S.A. (LUBS et al. 1977). In another study including Hawaiian orientals, Caucasians, Filipinos and Polynesians, significant differences were observed (MATSUURA et al. 1978). In chromosome No.1, the difference in heterochromatin size was entirely due to the larger size in Orientals, whereas in No.9 Caucasians had, on average, larger and Orientals smaller heterochromatic regions

than the other races. In No.16, no significant differences were found among the races although Caucasians possibly had a larger heterochromatic region than the other races.

Different definitions of the size variants by different groups of investigators may account for some of the reported differences in frequencies. From a general point of view, plus and minus variants appeared in roughly equal proportions in Nos. 1 and 9, probably with some slight excess of plus variants in No.1. In No.16, however, minus variants were much more common than plus variants, and in this respect, all reports are unanimous irrespective of the definition used or racial group studied. No.16 was also the chromosome with the highest incidence of variants.

Centromeric C-bands in chromosomes other than 1, 9 and 16 frequently differed in size between the white and black children (LUBS et al. 1977). The frequency of small bands was the same, but large bands were considerably more frequent in black children, most markedly in Nos. 4, 18, 19 and 22. Although not tested statistically, this indicates that there is a significant difference between the two races.

Concurrently with size variation, partial and complete pericentric inversions of heterochromatic regions are well documented in human chromosomes (CHAPELLE et al. 1974; MÜLLER et al. 1975; BUCKTON et al. 1976; LUBS et al. 1977; MAGENIS et al. 1977; METAXOTOU et al. 1978). Inversions seem to be most abundant in chromosome No.9, especially when complete inversions are considered (i.e. the whole heterochromatic region is located in the short arm). Complete inversions have not been reported in No.16 and partial inversions are also very infrequent in this chromosome. Similarly, complete inversions are much rarer in No.1 than in No.9. Again, significant racial differences in complete inversions of No.9 have been found between white and black children; such differences were more frequent in black children (LUBS et al. 1977).

Although the heterochromatic regions are liable to extensive variation in size and location, complete absence of C-bands is rare.

One case, however, has been described; a No.9 being devoid of detectable heterochromatin in a father and his child (BUYS et al. 1979). This condition occurs together with a completely normal phenotype, as is also the case with the size and location variants described above.

The studies of heterochromatin variation in European hedgehogs (I) revealed different modes of variation in three chromosome pairs. In two of them the variation was of a continuous nature, as in the human chromosomes 1, 9 and 16, whereas the third pair exhibits a discontinuous type of variation. Natural mouse populations also display size variants (FOREJT 1973) and this is mirrored by inbred mouse strains. Significant differences are recorded among several strains of different origins (FOREJT 1972, 1973; DEV et al. 1973; MÜLLER et al. 1976).

Variation within blocks of constitutive heterochromatin

After incorporation of BrdU into DNA during two rounds of replication and subsequent staining with the fluorescent dye Hoechst 33258 sister chromatids were found to fluoresce with different intensity (LATT 1973). It was soon noted that chromosomes of the mouse and of man could display differential staining of sister chromatids after only one round of replication in BrdU (LIN et al. 1974; LATT et al. 1974; GALLOWAY and EVANS 1975). Such simple lateral asymmetry was confined to regions of constitutive heterochromatin and was not seen in the remaining parts of the chromosomes.

In man, simple lateral asymmetry has been found in chromosomes 1, 15, 16 and the Y and possibly also in chromosomes 9, 17, 20, 21 and 22, although much less distinctly in these latter ones (ANGELL and JACOBS 1975; GALLOWAY and EVANS 1975; KIM 1975). In some individuals chromosome 1 displayed compound lateral asymmetry, i.e. both sister chromatids contained positively stained segments but with a maintenance of lateral asymmetry (GALLOWAY and EVANS 1975; ANGELL and JACOBS 1978; LIN and ALFI 1978). Variant patterns of Giemsa-11 positive bands were also detected within the C-band

region of chromosome 1 (MAGENIS et al. 1978). The patterns of these heteromorphisms were stable and were inherited in a simple Mendelian way. The centromeric heterochromatin of all mouse autosomes display simple lateral asymmetry; compound lateral asymmetry is found only in a few chromosome pairs in some strains, the pairs involved being different from one strain to another (JONASSON et al. 1979).

The simple lateral asymmetry has been interpreted as a reflection of a biased thymidine content in complementary strands of DNA, the compound asymmetry evolving from the simple by inversion. This kind of variants are thus most probably correlated with particular A-T rich segments of repetitive DNA.

The variable and complex composition of the heterochromatin of some human chromosomes is indicated by the staining with the G-C specific fluorochrome mithramycin (SIGMUND and SCHWARZ 1979). In chromosomes 1 and 16, but not in chromosome 9, the heterochromatic regions were subdivided into positively and negatively stained sections. In chromosome 1, the proportions of mithramycin-negative and -positive segments varied between chromosomes; the positive region being located proximally and the negative region distally within the heterochromatic block. Only occasionally, were the positive and negative regions intermingled.

Synthesis of variation of constitutive heterochromatin

Heterochromatin variants follow the rules of Mendelian inheritance in the vast majority of cases, as documented repeatedly in the studies of human variants (CRAIG-HOLMES et al. 1975; MCKENZIE and LUBS 1975; ROBINSON et al. 1976; CARNEVALE et al. 1976; PHILLIPS 1977). There exist some indications, however, that the exceptionally large heterochromatic region of chromosome 9 (9qh+) may undergo preferential segregation (FITZGERALD 1973; ROBINSON et al. 1976; CARNEVALE et al. 1976). Moreover, de novo variants, not present in the parents, have been reported in a few cases, as well as some cases representing mosaic conditions (CRAIG-HOLMES et al. 1975; MCKENZIE and LUBS 1975; NAKAGOME et al. 1977; PHILLIPS 1977).

The observations by CRAIG-HOLMES et al. that individuals may exhibit mosaicism for heterochromatin variants prompted them to suggest a mechanism of unequal crossing-over for the origin of the new size variants. Theoretically, unequal cross over products may be generated during mitotic, as well as meiotic processes. In mitotic cells, a mechanism similar to conventional crossing-over may be involved, or alternatively, unequal sister chromatid exchanges or amplification. The paucity of convincing evidence that chiasmata ever form in heterochromatic regions (JOHN 1976) disfavors the meiotic mechanism. In the European and the Algerian hedgehogs, no chiasmata are formed in the large heterochromatic regions (NATARAJAN and GROPP 1971). DNA synthesis during early meiotic prophase as demonstrated by HOTTA et al. (1966) and LIMA-DE-FARIA et al. (1968), was correlated to the recombination process. Absence of DNA synthesis during the pachytene stage in the satellite DNA sequences of man and mouse indicates that recombinational events are excluded from such regions (HOTTA and STERN 1978).

The occurrence of unequal exchanges or amplification in somatic cells is compatible with the finding of several new variants in cultured human fibroblasts after treatment with mitomycin C (HOEHN and MARTIN 1973), and with the cases of human mosaicism referred to above, showing both old and new variants. Whether some or all of the somatic mechanisms are operative, and if so, to what extent still remains obscure. In essence, however, it can be concluded that events giving rise to new heritable variants common to all cells of an individual, occur in the mitotic phase of germ line cells or in the premeiotic S phase, whereas, events giving rise to mosaic conditions occur in somatic cells during embryonic development.

New variants may also originate by simple exchanges between non-homologous chromosomes. This mechanism should only be effective in species having common repeated sequences distributed to several different chromosome pairs and located at the end of the chromosome as in the mouse. In such cases, simple exchanges of heterochromatic segments may take place, being facilitated by the participation of several homo- or heterologous chromosomes in chromocentric associ-

tion. Such a mechanism was proposed in Mus, to account for the variable distribution of NORs in different mouse strains (II; DI RARDINO et al. 1979). In some cases, unequal exchanges could occur, resulting in new variant sizes of heterochromatin.

new segments of constitutive heterochromatin visible as C-bands, may originate from sudden events of excessive replication of particular sequences - sudden as seen in an evolutionary time scale. This process was termed saltatory replication by BRITTEN and KOHNE (1969). FRY and SALSER (1977) found common sequences in satellite DNAs from various rodents. They suggested a new model, the library hypothesis, according to which rodents share a common library of satellite sequences. Certain members of the library, different in different species, are multiplied by saltatory replication, whereas other members occur in numbers below detectability.

reduction of heterochromatin may occur by simple deletion, even of large regions as in the Western European hedgehog (I). The geographical distribution of karyotypes having 2 or 3 chromosome pairs with heterochromatic blocks, is most compatible with the interpretation that the former originated from the latter by deletion of heterochromatic blocks in 1 pair.

What does variation of constitutive heterochromatin mean?

A great number of data on the properties of satellite DNA and heterochromatin have been scrutinized, and their possible functions discussed, in several recent papers (APPELS and PEACOCK 1978; ATHAK 1978; KURNIT 1979; JOHN and MIKLOS 1979; MIKLOS and JOHN 1979).

Numerous heteromorphisms and variants of constitutively heterochromatic regions have been recorded in various mammals, without noticing any obvious phenotypic effects. In humans, attempts have been made to find correlations between the presence of structural and size variants and malfunctions of any kind, as mental retardation, low IQ, psychiatric disorders, multiple malformations, increased inci-

dence of chromosomal aberrations, reproductive failure or fetal wastage and increased susceptibility to cancer. In most cases, no significant correlations were found, and among the few positive correlations discovered the causal connections, if any, were obscure. Thus, there seems to be a relationship between size heteromorphism and of pericentric inversion of 1qh and certain malignant diseases (ATKIN 1977; ATKIN and PICKTHALL 1977; ATKIN and BAKER 1977). In a group of 120 patients with malignant or premalignant diseases, SHANTAL and HALBRECHT (1979) found a significant incidence of variants in chromosomes 1 and 9. Patients with such variants usually had increased rate of chromosomal breakage and this chromosomal imbalance might be the basis for the initiation of malignancy.

In chromosome 9, complete pericentric inversion of the heterochromatic region was reported to be associated with reproductive failure (BOUE et al. 1975). The data of CHAPELLE et al. (1974) suggest, however, no increased rate of spontaneous abortions. Furthermore, NIELSEN et al. (1974) found indications of an increased risk of chromosome abnormalities in the progeny of individuals with 9qh+ variants.

Cytogenetical analysis of the Australian grasshopper genus Atractomorpha demonstrated that the heterochromatin affects the chiasma formation (MIKLOS and NANKIVELL 1976). The 3 species studied differed with respect to the presence or absence of centromeric and terminal heterochromatin. Chiasmata were localized away from the heterochromatin and when heterochromatic segments were present at both ends, the chiasmata formed at a maximum distance from both segments. The proportion of multiple chiasmata decreased when the number of heterochromatic segments increased and consequently the overall chiasma frequency was reduced.

The same phenomenon, localization of chiasmata away from the heterochromatin, was observed before in the Desert locust by FOX et al. (1973). They suggested that this was a general phenomenon and a mechanism for maintaining supergenes. FOREJT (1973) suggested that size polymorphisms of the heterochromatin in mice might suppress the genetic recombination close to the heterochromatic seg-

ments. Such ideas have been outlined in more detail by ÁRNASON et al. (1978). They argue that microscopically detectable C-band heteromorphisms must create mechanical difficulties for meiotic pairing, resulting in a mismatch close to the C-band, but that intimate pairing will eventually be restored along the length of the chromosome. The genetical consequence of this mechanism would be that the blocks close to the heterochromatic segments are preserved. This will create genic polymorphism within a population and eventually promote speciation.

With this mechanism in view, hybrids between closely related species, differing only in amount and/or distribution of constitutive heterochromatin in several chromosome pairs, could be expected to suffer from meiotic disturbances, resulting in univalents and ultimately in unbalanced gametes.

A number of other roles have been ascribed to heterochromatin, as initiators of homologous pairing, inert sites for chromosome breakage facilitating structural rearrangements, a protective shield against damage of the euchromatin (bodyguard hypothesis), aiding centromeric strength and so forth. It may be fruitless to search for the function of heterochromatin. Qualitatively different kinds of heterochromatin, as well as localization to different sites within a chromosome, may predispose to different functions. Some types of heterochromatin may have lost their function, being candidates for elimination.

ACKNOWLEDGMENTS - I would like to express my sincere gratitude to Carl Fredga for his personal interest in my work and scientific education and for his inspiring collaboration. I also wish to thank Albert Levan and Karl Fredga for their guidance and encouragement over the past years and for their constructive criticism of my manuscripts. I am also grateful to Arne Lundqvist for his indispensable help in the final preparation phase of this thesis.

I wish to heartily thank numerous colleagues and friends for handling my tissues, typing manuscripts, collecting and tending experimental animals, helping with photographic reproduction, discussions and above all for their stimulating and enjoyable companionship.

Financial support from the Swedish Natural Science Research Council, the Carl Trygger foundation, the Nilsson-Ehle Foundation the Swedish Cancer Society and the Heinrich Poll Genetical Scholarship is gratefully acknowledged.

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A comparative chromosome study by means of G-, C-
and NOR-bandings of the weasel, the pygmy weasel
and the stoat (Mustela, Carnivora, Mammalia).

by

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1979



ABSTRACT

Mandahl, N. and Fredga, K. 1980. A comparative chromosome study by means of G-, C- and NOR-bandings of the weasel, the pygmy weasel and the stoat (Mustela, Carnivora, Mammalia). - Hereditas 93:00-00. Lund, Sweden. ISSN 0018-0061
Received October 12, 1979

The karyotypes of 9 weasels (Mustela nivalis vulgaris), 7 pygmy weasels (M. n. nivalis) and 11 stoats (M. erminea) were studied. G-, C- and NOR-banding revealed distinct similarities between the species/subspecies. The two weasel forms have $2n=42$ and identical karyotypes, the stoat has $2n=44$. The weasel karyotypes differ from the karyotype of the stoat by one centric fusion and by the addition of large blocks of constitutive heterochromatin to the short arms of 7 chromosome pairs. Only one chromosome pair in each species has a nucleolar organizing region.

One weasel and one stoat had aberrant karyotypes. The weasel had extra chromosome material of unknown origin added to one chromosome, the stoat had $2n=43$ due to centric fusion of two t chromosomes.

The species problem in Scandinavian weasels and karyotype evolution in the Mustelinae are discussed.

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INTRODUCTION

The initial purpose of the present study, started in 1966, was to find out whether comparative chromosome studies could cast some light on the species problem of Scandinavian weasels. The two forms of weasels, by some regarded as separate species, differ in size, pelage characters and distribution. Conventional orcein staining revealed no difference in the karyotypes of the two forms (Fredga 1967).

When the chromosome banding techniques were introduced, permitting much better resolution, we decided to consider the problem again. Autoradiography and C-banding revealed that large heterochromatin blocks were present in several autosome pairs of the pygmy weasel (Fredga and Mandahl 1973). Since heterochromatin is known to show great variation in size and location, not only between species and subspecies but also among individuals of the same population, and even between the two homologues of a chromosome pair, we realized that the material had to be extended to more than just a few specimens of each type. We also realized that it should be possible to compare in a meaningful way the seemingly very divergent karyotype of the stoat with those of the weasels. The results of the comparative chromosome studies with G-, C- and NOR-banding techniques in the two types of weasels and the stoat are presented below. Two apparently healthy individuals trapped in the wild, one weasel and one stoat, had aberrant karyotypes, not explainable by C-band variation.

MATERIAL AND METHODS

Eleven stoats (Mustela erminea) and 9 weasels (provisionally Mustela nivalis vulgaris) were all trapped in southern Sweden, in the province of Scania, and 7 pygmy weasels (provisionally M. nivalis nivalis) were trapped along their entire range of distribution in northern Sweden. (cf. Table 1)

In the present paper all animals successfully studied during a period of 13 years are included. For this reason several specimens have not been studied by chromosome banding techniques. The tissues and staining techniques used are summarized in Table 1. Some of the specimens were included in two earlier reports (Fredga 1967; Fredga and Mandahl 1973).

The cell culture, chromosome preparation, and staining techniques have been described previously (Fredga and Mandahl 1973; Mandahl and Fredga 1975). For G-bands the trypsin - Giemsa technique of Wang and Fedoroff (1972) was used and for C-bands the Ba(OH)_2 - Giemsa technique of Sumner (1972). For selective staining of the nucleolar organizing regions (NORs) the Ag-I technique of Bloom and Goodpasture (1976) was applied.

RESULTS

The chromosomes of the weasel and the pygmy weasel were arranged in the karyotypes in decreasing size order according to Fredga and Mandahl (1973). The chromosomes of the stoat were arranged so as to facilitate comparisons between the karyotypes of the weasels and the stoat and not according to the size criterion.

The weasel and the pygmy weasel had numerically ($2n=42$) and morphologically identical chromosomes, most of them being of the m or sm type (nomenclature according to Levan et al. 1964). The stoat had $2n=44$ and a greater number of st and t chromosomes. All specimens of weasels and stoats usually had a very prominent secondary constriction in the proximal part of the long arm of a medium-sized chromosome (No. 14). The sex chromosomes were of the m type and of the same size in all three species/subspecies. The X chromosome was of the original type and the Y chromosome was the smallest member of the chromosome sets.

G-staining

The G-band patterns of the chromosomes of the two weasels and the stoat permitted unambiguous identification of all chromosome pairs. The band patterns of the weasel and the pygmy weasel were identical (Fig. 1a and 2a). Both had 6 pairs (Nos. 1, 2, 5, 6, 7 and 8) of autosomes with large homogeneously weakly stained regions constituting the major and distal part of their short arms. A seventh pair (No. 13) had a smaller short arm with the same staining properties. The secondary constriction of No. 14 was unstained.

The band patterns of the chromosomes of the stoat (Fig. 3a) and the weasels were very similar. The main differences consisted in the total, or almost total, absence in the stoat of the large weakly stained regions, the largest segment of this kind being found in chromosome No. 1. In addition, the large m chromosome No. 4 of the weasel karyotype was represented by two t chromosomes in the stoat. In their band patterns the two t chromosomes corresponded perfectly to the two arms of the m chromosome No. 4 and were consequently designated 4p and 4q, respectively.

C-staining

In the weasel and the pygmy weasel 7 chromosome pairs had large blocks of C-band positive material (Fig. 1b and 2b). These blocks corresponded to the weakly G-stained short arms of chromosomes 1-2, 5-8 and 13. In these chromosomes a small, less darkly stained region was sometimes seen in the proximal part of the short arm, especially in chromosome Nos. 1, 2 and 5, but usually the C-block and the centromeric C-band coalesced. The size of the C-blocks varied in several pairs of different specimens.

All chromosomes had centromeric C-bands, the sizes of which also varied somewhat from one animal to another. The largest bands were found in chromosome Nos. 3, 4, 9, 10, 14, 18 and 20.

Interstitial bands were present in the proximal half of the short arm of No. 4 and in the region of the secondary constriction of No. 14. These bands were more weakly stained than the C-blocks and the centromeric bands and were less regular in appearance. In some animals, a narrow and faint terminal band was observed in the short arms of Nos. 10 and 11. About 3/4 of the Y chromosome was C-band positive; only the distal part of the short arm stained weakly. No significant differences in C-band pattern were observed between the weasel and the pygmy weasel.

The stoat chromosomes had, in contrast to the weasels, no large C-blocks at all (Fig. 3b). Distinct C-bands were found in all centromeric regions. The small short arms of chromosome Nos. 1, 2, 5, 6, 7, 8 and 13 were partially or completely C-stained. Since, in the stoat, Nos. 1, 2, 10 and 11 were quite similar in size and shape, their identity was established only by consecutive G- and C-staining of the same metaphases. In Nos. 10 and 11 of the stoat there were distinct terminal bands in the short arms, which in the weasels were much smaller and were only found in a few animals. Both in the weasels and in the stoat, a weak band was occasionally noticed close to the secondary constriction of No. 14. No band corresponding to the interstitial band of No. 4 of the weasels was ever observed in the stoat. The Y chromosome was similar to that of the weasels.

Ag-I staining

It was established by Ag-I staining that only one chromosome pair had active ribosomal genes in the two weasels and the stoat (Fig. 4). Not unexpectedly the Ag-NORs were located in the prominent secondary constriction of chromosome No. 14. No other sites were detected. In some cells the silver stained region was very large. When the preparations were incubated for too long a time with AgNO_3 -solution the large heterochromatic blocks in the chromosomes of the weasels became stained more intensely than the rest of the chromosomes, just as mouse centromeres tend to do.

Abnormal karyotypes

Two aberrant karyotypes were found among the 27 specimens studied, one in a weasel and one in a stoat. The weasel in question had the normal diploid chromosome number of 42, but in pair No. 9, one homologue was structurally abnormal, being a large m chromosome instead of a medium-sized sm chromosome (Fig. 5). The aberrant m chromosome was present in all metaphases analyzed in direct preparations from bone marrow and in preparations from tissue cultures of lung, skin and testis. In C-banded metaphases the m chromosome displayed a C-band positive region covering the distal 2/3 of the short arm. The staining was, however, less intense than in the regular C-blocks. Since the C-block of one chromosome No. 6 was unusually small, a translocation of heterochromatin from No. 6 to No. 9 was considered as a possible explanation for the increased size of No. 9. However, the G-band pattern did not support this idea. The extra material in the short arm of No. 9 did not show the homogeneous weak staining characteristic of the C-block regions, but was differentiated into dark and light bands. The G-band pattern (but not the C-band pattern) of the whole changed arm of the abnormal No. 9 closely resembled that of the short arm of No. 4; the long arm of No. 9 was unchanged. In the other chromosomes there was no segment missing and there were no other peculiarities that could give a clue about the origin of the extra material in No. 9.

The other case of an aberrant karyotype was found in a stoat. The chromosome number was 43 and a large m chromosome not seen in the normal stoat karyotype was present. By G-banding it could be demonstrated that this chromosome had originated by centric fusion of chromosome Nos. 4q and 7 (Fig. 6). Unfortunately, no direct preparations were available from this animal and only one tissue (lung) was studied. The occurrence of the m chromosome in every analyzed cell from the first passage, i.e. about 10 days after initiation of the culture, strongly indicates that it was present in the live animal and not produced in vitro.

In the tissue cultures from another stoat, a new large m chromosome appeared. The original karyotype was normal, but after approximately 10 weeks in culture, cells with the m chromosome predominated. It was composed of chromosomes 2 and 4p joined by a centric fusion.

DISCUSSION

The taxonomic relations of the 2 weasel forms

The question whether the weasel and the pygmy weasel should be ranked as different species or as subspecies has been a matter of debate (Siivonen 1968; Ewer 1973; Corbet 1978; Stolt 1979). The specific status has been favoured by Siivonen and by Ewer. Corbet lists only one species, Mustela nivalis, and Stolt arrived at the provisional conclusion that they represent two subspecies in Sweden, M. n. vulgaris and M. n. nivalis.

The primary aim of this study was to make a detailed chromosome analysis of the weasel and the pygmy weasel in order to find out whether data from this field of investigation could add any essential information to this discussion. The analysis of the chromosomes of the two weasels by banding techniques led to the conclusion that their banding patterns do not differ in any respect. The G-band patterns were identical as well as the localization of Ag-NORs. Although C-band polymorphism was observed, the distribution of dark C-bands did not differ between the two weasels.

Both forms of weasels have been found in a 100 km wide overlapping zone, running through central Sweden (Stolt 1979). Hybrids possibly formed within this zone should not suffer from meiotic disturbances due to morphological differences of the chromosomes. This, however, does not exclude that hybrid sterility or subfertility may occur for other reasons. The 332 weasels analyzed by Stolt, contained no single specimen that could be classified as a true hybrid.

This problem has also been dealt with by Fritz Frank in Braunschweig, Germany. He succeeded in breeding typical representatives of the two forms trapped in Sweden, and he also proved that the F₁ offspring was fertile (Frank, personal communication). Thus, the problem seems to be solved, at least as far as Swedish weasels are concerned: the larger southern weasel and the smaller northern weasel belong to the same biological species. The chromosomal data of the present study rather supports this conclusion.

The 7 heterochromatic blocks in the weasels are presumably composed of repetitive DNA sequences as indicated by positive C-staining. Light G-staining and negative fluorescence after Q-staining (Fredga and Mandahl 1973) indicate a relatively high content of GC rich DNA. Detailed studies with biochemical techniques might possibly reveal differences in DNA composition between the two weasels.

The karyotype evolution in the Mustelinae

All species of the subfamily Mustelinae so far studied, excepting the American mink (Mustela vison), have diploid chromosome numbers ranging from 38 to 44 (Matthey 1973). The American mink has a distinctly lower number, viz. $2n=30$. In spite of the large numerical difference between the stoat and the American mink, a few chromosomes and still more chromosome arms have identical G-band patterns (Grafodatsky et al. 1976). For instance, No. 14 of the weasels and the stoat, carrying the NOR corresponds exactly (G-, C- and Ag-I staining) to the short arm of a medium-sized m chromosome (No. 8 in Mandahl and Fredga 1975), which is one of the two pairs with NORs in the American mink.

According to Grafodatsky et al. (1976), the G-band patterns of 7 species of mustelids indicated that the karyotype of the stoat represents the most original one. Furthermore, Grafodatsky et al. (1976, 1977) suggest that the karyotype evolution of the mustelids proceeds by decrease of the number of acrocentrics by Robertsonian fusions and by increase of the amount of heterochromatin. Other kinds of changes also occur, although they are of minor significance. The sex chromosomes and some autosomes are conserved with an unaltered G-band pattern in all species studied.

The karyotypes of the two weasels on the one hand and the stoat on the other, display morphological dissimilarities in several chromosome pairs, as well as the difference in chromosome number ($2n=42$ and 44 , respectively). The karyotypes of the weasel and the pygmy weasel can easily be derived from the stoat chromosomes simply by adding heterochromatin to the short arms of 7

chromosome pairs (Nos. 1, 2, 5, 6, 7, 8 and 13) and by fusing two pairs of t chromosomes (Nos. 4p and 4q). Some other differences do exist. In the chromosomes of a few of the weasels Nos. 10 and 11 have minute terminal C-bands in the short arms, whereas the corresponding chromosomes in all stoats have distinct bands. Furthermore, the interstitial C-band in the short arm of No. 4 present in the weasels was not observed in the stoat chromosomes. Thus, both gain and loss of C-positive material have been involved, while the G-band pattern has remained essentially unchanged.

The mountain weasel, Mustela altaica, has a karyotype sharing characteristics with the karyotypes of the weasel and the stoat. In conformity with the weasel, the mountain weasel has 7 large heterochromatic short arms (Grafodatsky et al. 1977), and in agreement with the stoat, has 44 chromosomes. The large m chromosome without C-block (No. 4), present in the weasel, is absent in both the mountain weasel and the stoat. Large terminal C-blocks are also found in the European polecat (Mustela putorius) and the steppe polecat (Mustela eversmanni) (Fredga and Mandahl 1973; Grafodatsky et al. 1977). However, in these two species large C-blocks are present only in 3 and 1 chromosome pairs, respectively. Other musteline species apparently lack large terminal blocks of constitutive heterochromatin.

Karyotypic deviations of single individuals

Abnormal karyotypes were found in two animals in our material, viz. in one weasel and one stoat. The origin of the extra material in the short arm of one chromosome, No. 9, in one of the weasels, remains obscure. No part of any other chromosome was missing; only C-band heteromorphisms in Nos. 6 and 13 were present. The staining properties of the extra material, being weakly C-band positive and differentially stained by G-banding, contradict the possibility that it represents the same kind of heterochromatin (heavily C-band positive and homogenous weak G-staining) that is found in the short arms of chromosome Nos. 1, 2, 5, 6, 7, 8 and 13. No obvious phenotypic malformations or permutations were noticed in this animal.

In the stoat, one of the t chromosomes, No. 4q, that corresponds to the long arm of the large m chromosome No. 4 in the weasels, was translocated to the short arm of No. 7. Another translocation, between the t chromosomes 4p and 2, was found frequently in cells of passage 18 of tissue cultures from another stoat. The original karyotype was normal. Both cases involve one of the t chromosomes that occur fused in the weasels and a chromosome that has large C-blocks in the weasels. This may be a coincidence but might well indicate an increased tendency of these t chromosomes to participate in Robertsonian translocations.

Acknowledgements. - We gratefully acknowledge the receipt of living animals from Ulf Bergström, Sam Erlinge, Ingrid Hansson, Lennart Hansson, Janerik Nawrin, Anders Rönnow and Artur Åkesson. We thank Ulf Wiberg and Bengt Widegren for assistance during trapping. Our sincere thanks are also due to Albert Levan and Penny Kenton Russ for critically reading the manuscript.

This work was supported by grants from the Swedish National Science Research Council and the Royal Physiographic Society in Lund.

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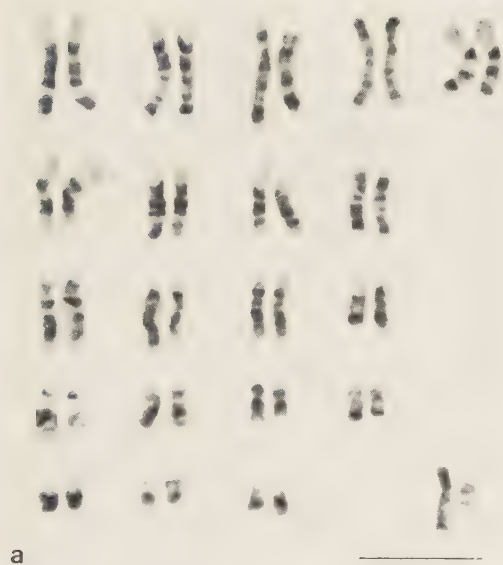
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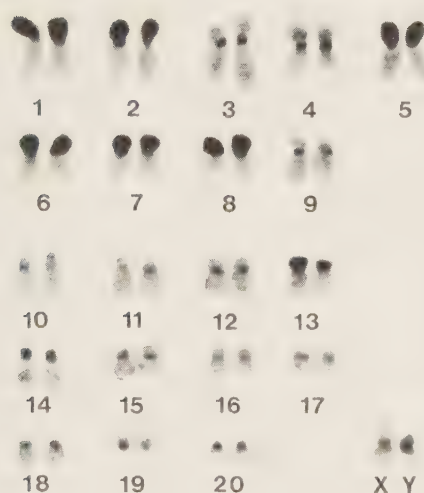
Table 1. Survey of the material. All animals studied are from Sweden. The following abbreviations are used: Tissues: BM = bone marrow, Bl = Blood, Lu = Lung, He = Heart, Sk = Skin, Te = Testis; C = chromosomes counted in the microscope, K = chromosomes karyotyped. Staining techniques: O = orcein or Giemsa without banding, N = selective staining of NORs with Ag-I, AO = acridine-orange after denaturation-renaturation, G, C, Q and R according to Paris Conference (1971), A = autoradiography.

Animal No.	Sex	Tissue				Chromosomes		Place	Trapped in Province	Year	Collectors
		BM	Bl	Iu	He	Sk	Te				
Weasel 1	♂	K		K	C			Ebbesjö	Skåne	1965	A. Rönöw
2	♀	C		K	K			S. Sandby	"	1964	I. Hansson
3	♀			C	C			Kullaberg	"	1965	L. Hansson
4	♂			K	C			Stensoffa	"	1965	"
5	♀			C	K			Kullaberg	"	1966	"
6	♂	K		K	C			"	"	1964	"
7	♀	K		C	C			Dalby	"	1964	"
8	♂	C		K		K	K	Skarhult	"	1979	K. Fredga, N. Mandahl
9	♂		C	K	K		K	Stensoffa	"	1979	N. Mandahl
Pygmy weasel 10	♀	C		K	K			Pajala	Lappland	1965	I. and L. Hansson
11	♂			K	C			-	Jämtland	-	U. Bergström
12	♂	C		K	C			Arvidsjaur	Lappland	-	"
13	♂			K				NE Västerås	Västmanland	1974	J. Nawrin
14	♀			K				Hällnäs	Västerbotten	1974	"
15	♂			K				Garpenberg	Dalarna	1974	N. Mandahl, J. Nawrin
16	♀				K			Bispgården	Jämtland	1977	K. Fredga
Stoat 17	♀	K		K	K			Skarhult	Skåne	1966	A. Åkesson
18	♀			C	C			Kullaberg	"	1966	L. Hansson
19	♀	K		C	C			S. Sandby	"	1966	"
20	♂			C	K			Skårby	"	1968	K. Fredga
21	♀	K		C	C			S. Sandby	"	1964	I. Hansson
22	♂			C				Skarhult	"	1975	S. Erlinge
23	♂			K				"	"	1975	"
24	♂			K				"	"	1975	"
25	♂			C				"	"	1975	"
26	♂			K				"	"	1975	"
27	♂			K				Stensoffa	"	1975	"

Additional chromosome material in one homologue of pair No. 9. ²Robertsonian fusion of one member of pairs Nos. 4q and 7.

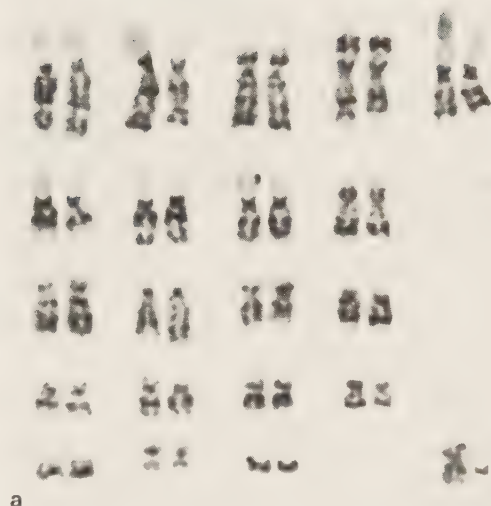


a

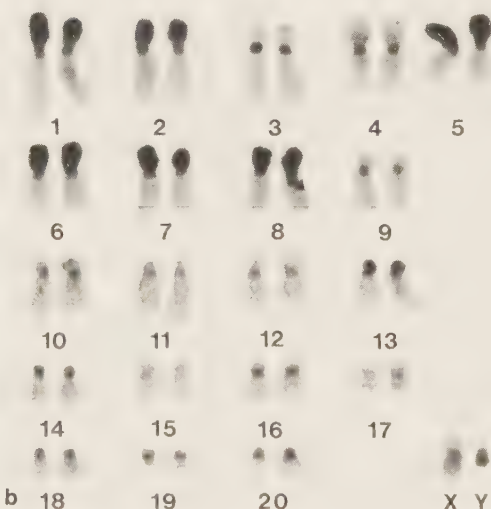


b

Fig. 1 Banded karyotypes of the weasel, a) G-staining; b) C-staining. Both karyotypes, from the same specimen, display heteromorphism in the short arm of chromosome 13. - Scale indicates $10\mu\text{m}$.



a



b

Fig. 2 Banded karyotypes of the pygmy weasel, a) G-staining; b) C-staining, from different specimens. The short arms of chromosome 5 are heteromorphic. - Scale indicates $10\mu\text{m}$.

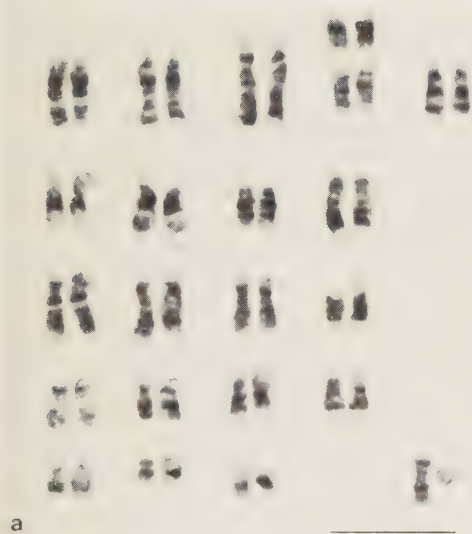


Fig. 3 Banded karyotypes of the stoat, a) G-staining and b) C-staining. Both karyotypes are from the same specimen. Two t chromosomes, 4p and 4q, are placed together in order to facilitate comparison with the chromosomes of the weasels. - Scale indicates 10 μ m.

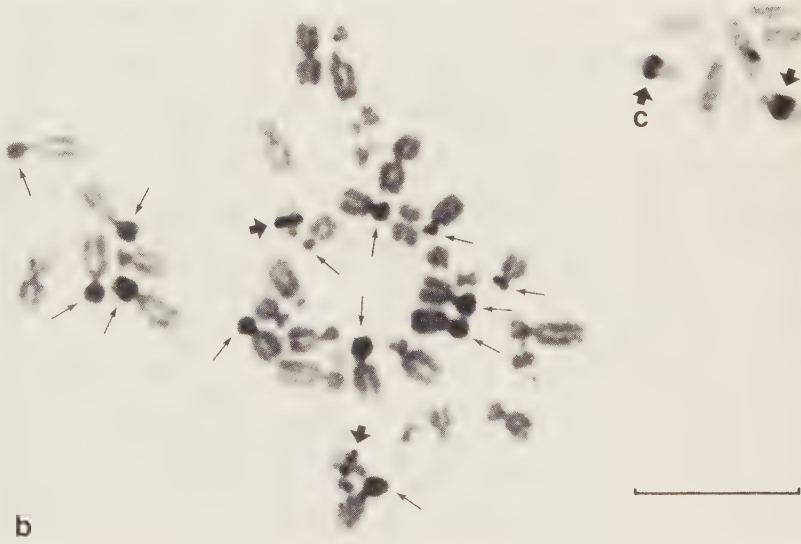
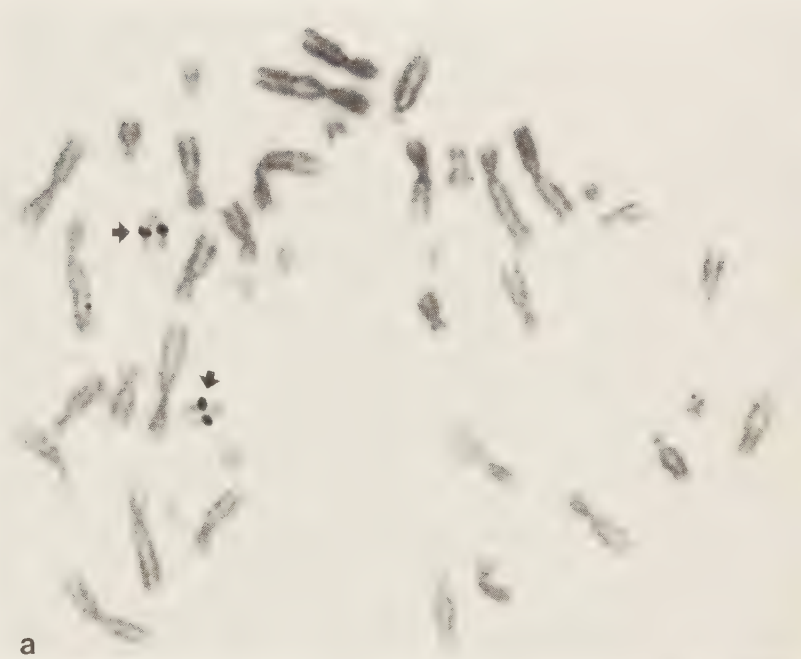
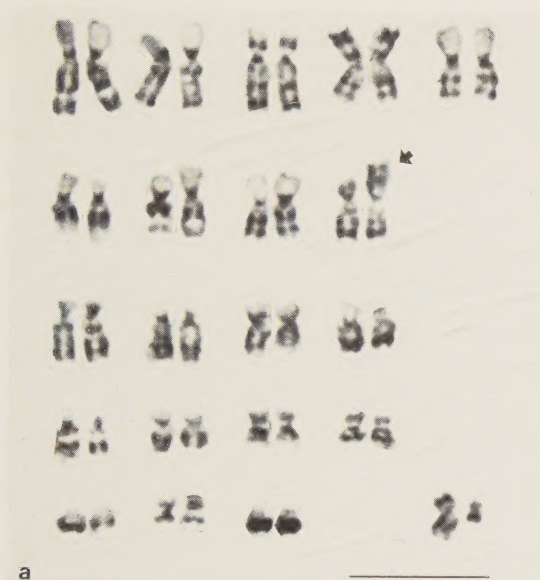
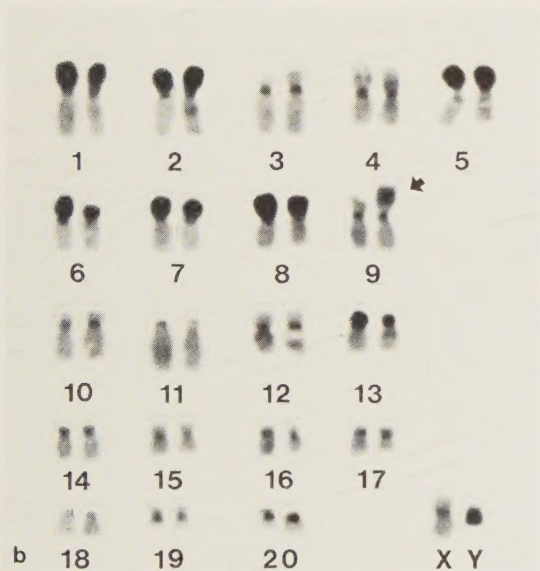


Fig. 4 Silver staining demonstrating the localization of NORs. The weasel (a, b), the pygmyweasel, and the stoat (c) all have Ag-NORs only in one chromosome pair. a) Ag-NORs indicated by arrows; b) incubation in AgNO₃ solution exceeding the optimal time results in dark staining both of the NORs (thick arrows) and of the heterochromatic short arms (thin arrows); c) very large silver precipitates were observed in some cells. - Scale indicates 10 μ m.

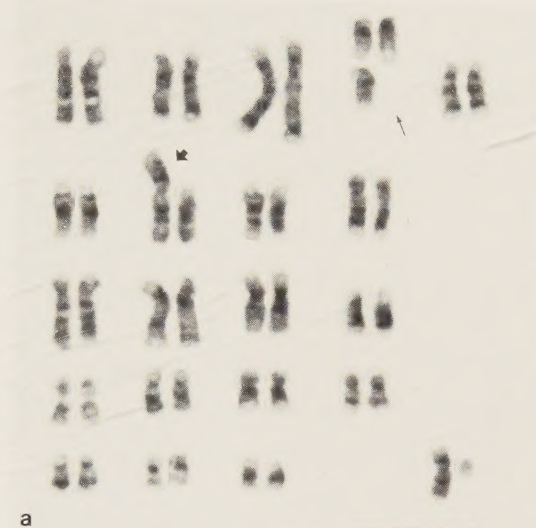


a



b

Fig. 5 G- and C-banded karyotypes of the chromosomally abnormal weasel. One homologue of chromosome 9 (arrow) contains extra material in the short arm. Note the less intense C-staining of the additional material, and also evident heteromorphisms in the C-blocks of chromosomes 6 and 13. – Scale indicates $10\mu\text{m}$.



a



b

Fig. 6 G- and C-banded karyotypes of the stoat having $2n=43$, due to a Robertsonian fusion. The large m chromosome composed of 4q and 7 is indicated by a thick arrow and the absence of one t chromosome by a thin arrow. – Scale indicates $10\mu\text{m}$.

